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Mycobiota of Wheat Seeds with Signs of "Black Point" under Conditions of Forest-Steppe and Forest Zones of Ukraine †

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Abstract: Composition of phytopathogens—causal agents of "black point" was studied in 2018–2019. Investigations were carried out at the Institute of Plant Protection of National Academy of Agrarian Sciences of Ukraine. Seed samples were taken from different locations in the Forest-Steppe and Polissya zones of Ukraine. The results obtained showed that fungi from the genus Alternaria Nees dominated in the pathogen complex. They were isolated from 76.5–83.1% of seeds from the Forest-Steppe of Ukraine. Less often they were found in samples from the Polissya zone (59.0–69.4%). Alternaria tenuissima та Alternaria infectoria were isolated in an almost equal proportion. In addition, seeds with "black point" were colonized by fungi from genera *Fusarium* Link, *Curvularia* Boed, *Bipolaris* Shoem., *Aspergillus* P.Micheli, *Nigrospora* Zimm., *Cladosporium* Link, *Acremoniella* Sacc., *Stemphillium* Walr., *Sordaria* Ces. & De Not, *Epicoccum* Link, *Aureobasidium* Viala at Boy. It should be noted that the causal agent of nigrospora dry rot of maize *Nigrospora oryzae* (Berk & Broom) Petch was isolated from nearly 6% of seeds from the Polissya zone. In addition, saprophitic fungi *Stemphillium* spp. and *Sordaria* spp. were found. The majority of isolated fungi produce mycotoxins which are harmful for people and animals. Thus, the monitoring of species composition of pathogens on wheat seeds, in particular causal agents of "black point", is essential for obtaining a high-quality wheat grain.

Keywords: wheat; black point; composition of phytopathogens; *Alternaria* spp.; *Nigrospora oryzae*

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1. Introduction

In Ukraine, wheat is grown on about 6.6 million hectares, which is 27% of field crops. The yield of this crop usually depends on weather conditions, agricultural techniques and plant protection systems and in last 20 years averages 20 to 40 dT/ha [1]. Winter wheat is mainly grown. The main crops are concentrated in the central and southern part of the country.

The quantity and quality of the harvest also depends on the development of useful and pathogenic microflora on the ear and grain, respectively. About 100 phytopathogens of fungal etiology are known, which can colonize the grain during ripening [2]. Most often it is *Fusarium* spp., *Alternaria* spp., *Bipolaris* spp. The following fungi can also infect grain: *Cladosporium* spp., *Rhizopus* spp., *Curvularia* spp., *Aspergillus* spp., *Stemphylium* spp., *Penicillium* spp., *Chaetomium* spp. and others.

Infection can manifest itself as the appearance of a dark color of the grain in the area of the embryo. Most studies in the world have found that the main reason for the appearance of dark-colored germ in wheat is the penetration of fungi *Alternaria* spp. and *Bipolaris* spp. in the germ area during grain filling. This causes discoloration of the endosperm tissues of the seed and the growth of pathogenic mycelium inside it, which is a diagnostic sign of the disease [3].

The aim of our investigations was to determine the phytopathogenic composition of "black point" pathogens and their share in winter wheat seeds in the Forest-Steppe and Polissya zones of Ukraine.

2. Materials and Methods

The research was conducted in 2018–2019 at the Institute of Plant Protection of the National Academy of Agrarian Sciences of Ukraine. Seeds selected from batches of winter wheat grown in Kyiv (Forest-Steppe) and Chernihiv (Polissya) regions of Ukraine were used for analysis. In 2018, 31 seed samples from the Kyiv region and 22 from the Chernihiv region were analyzed, in 2019—27 and 21 samples, respectively. About 600 fungal isolates were analyzed to identify the species composition and establish their share in the phytopathogenic complex of winter wheat seeds with "black point".

Seeds with symptoms of "black point" were selected from each sample. The seeds were washed with running water for 2 h. Surface sterilization was performed with 96% ethyl alcohol for 1 min. Sterilized seeds were dried between layers of sterile blotter paper. After that, the seeds were placed on a nutrient medium with the addition of antibiotics (gentamycin). Potato carrot medium (PCM) was used to identify fungi of the genus *Alternaria*. Incubation was performed at a temperature of 22–25 °C under fluorescent lamps. Fungi were identified on 10–14 day. Species of the genus *Alternaria* was determined by several parameters: morphological and cultural characteristics, sporulation habit and size, structure of conidia [4]. Other species were identified by morphological characteristics of conidia using a microscope [5,6].

3. Results

In the years of investigation, weather conditions during the ripening of winter wheat were favorable for grain infection and development of infection in the germ area. As a result, the appearance of seeds with signs of darkening in the embryonic zone was observed.

In 2018, both the Forest-Steppe zone and Polissya were characterized by a high level of moisture during grain filling and harvesting (Figures 1 and 2). In the Kyiv region, the hydro-thermal coefficient (HTC) in June was 1.8 and in July was 1.4. In the Chernihiv region, it was, respectively, 2.3 and 2.0.

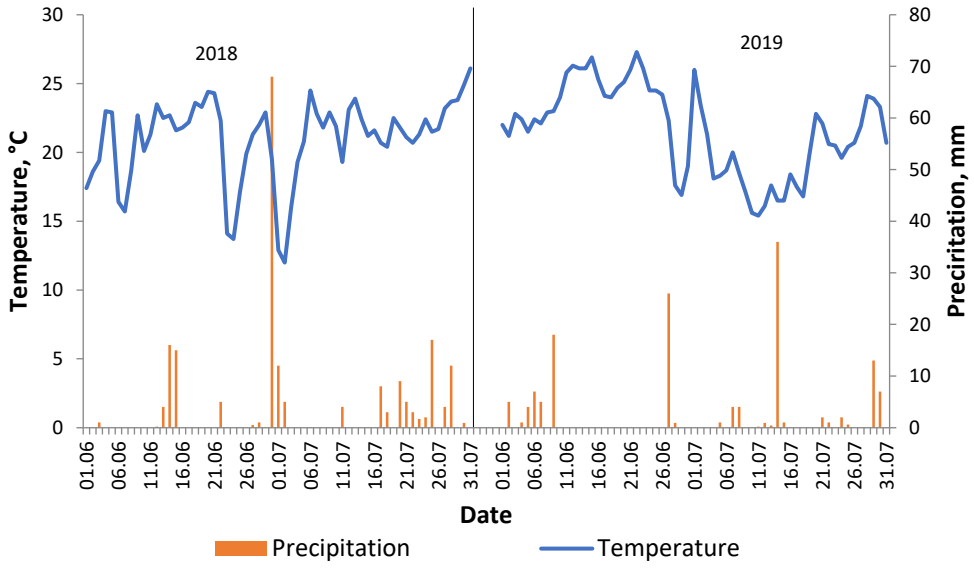

Figure 1. The weather conditions in June–July in the Kyiv region (2018–2019).

In 2019, the level of humidity in this period in the Kyiv region was lower—HTC = 1.2 for June–July. Under the conditions of the Chernihiv region, a dry June (HTC = 0.3) and rather wet July (HTC = 1.13) were observed.

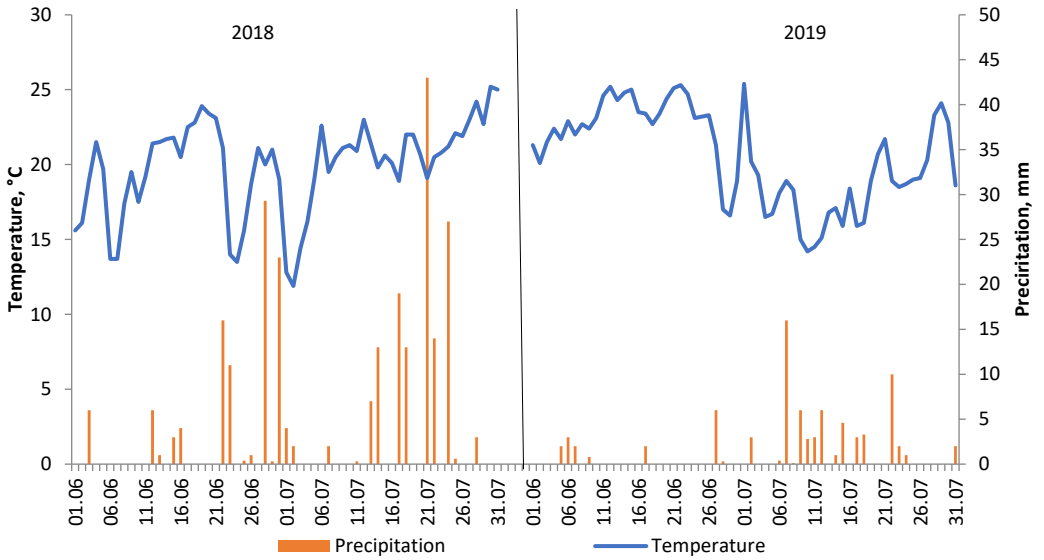

Figure 2. The weather conditions in June–July in the Chernihiv region (2018–2019).

An analysis of seeds with signs of "black point" from the Forest-Steppe (Kyiv region) for pathogenic microflora revealed significant contamination by fungal pathogens and insignificant development of bacterial infection. The phytopathogenic complex in the Forest-Steppe included 13 species of fungi from 9 genera. In the Polissya zone (Chernihiv region), 11 species of fungi from 8 genera were isolated during the research years. Fungi from the genera *Alternaria*, *Fusarium*, *Curvularia*, *Helminthosporium*, *Aspergillus*, *Acremoniella*, *Stemphillium*, *Sordaria*, *Epicoccum*, *Nigrospora*, *Cladosporium*, *Aureobasidium* were found (Table 1). Bacterial infection was recorded mainly in seed samples from the Polissya area, their share was 4.1% (2018) and 0.5% (2019), in the forest-steppe was detected only in 2019 at 1.5%.

Table 1. Mycobiota of winter wheat grain with "black point" from the Forest-Steppe (Kyiv region) and Polissya zones (Chernihiv region) in 2018–2019.

Species	Isolation Ratio, %			
	Forest-Steppe Zone		Polissya Zone	
	2018	2019	2018	2019
Alternaria	76.5	83.1	69.4	59
Including *Alternaria tenuissima*	37.5	41.5	31.5	27.1
Alternaria infectoria	39	35.4	37.8	31.4
A.alternata	0	2.4	0	0.5
Alternaria sp.	0	3.8	0	0
Fusarium spp.	3.1	0.8	4.7	10.6
Curvularia spp.	1.6	0	0	0
Bipolaris sorokiniana	0	1.5	3.5	4.8
Nigrospora oryzae	0	0	5.9	5.8
Aspergillus spp.	0	4.6	0	0
Acremoniella atra	0	0.8	0	1.6
Stemphillium vesicarium	4.7	1.5	2.9	3.7
Sordaria fimicola	7.8	3.8	2.3	6.4
Epicoccum nigrum	0	2.4	3.5	2.6
Cladosporium herbarum	0	0	0	1.1
Aureobasidium pullulans	0	0	0.6	0.5
Sterile mycelium	6.3	0	3.7	3.4
Bacterial infection	0	1.5	4.1	0.5

Fungi of the genus *Alternaria* (*A. tenuissima*, *A. infectoria*, *A. alternata*) were most often isolated from seeds with black germ in both study areas, the share of which was 76.5% in the Kyiv region in 2018 and 83.1% in 2019. In the Chernihiv region, the black germ of Alternaria origin was found on 69.4% of seeds in 2018 and 59% in 2019.

The share of other species varied depending on the region and year of research. In the Forest-Steppe zone it was in total 23.5% in 2018 and 15.4% in 2019. In the Polissya zone, it was 26.5% in 2018 and 40.5% in 2019.

4. Discussion

Alternaria spp. and *Bipolaris* spp. are the main causative agents of darkening in the germ zone of cereals. Kai-Ge Xu et al. [6] showed that the most common pathogens of "black point" in China are *Alternaria* (56.7%), *Bipolaris* (16.1%) and *Fusarium* (6.0%). The frequency of detection of fungi from other genera (*Curvularia*, *Aspergillus*, *Cladosporium*, *Exserohilum*, *Epicoccum*, *Nigrospora*, *Penicillium* and *Ulocladium)* ranged from 0.8 to 4.8% [7]. Fungi of the genus *Alternaria* (49.9%), and to a lesser extent *Aspergillus* (26.5), *Drechslera* (11%), *Fusarium* (7.9%), *Cladosporium* (3.8%) and *Curvularia* (0.7%) were also found in Pakistan [8].

In Argentina, fungi of the genus *Alternaria* were found to be the main causative agent of the "black point", they were found on 76–85% of seeds. The incidence of *Bipolaris sorokiniana* was 9–13% [9].

Our investigations show that fungi of the genus *Alternaria* are also the main causative agents of "black point" in Ukraine. In the Forest-Steppe zone in 2018, two species of this genus *Alternaria tenuissima*—37.5% and *Alternaria infectoria*—39% were identified on wheat grain with black germ. In 2019, 4 species were identified: *Alternaria tenuissima*—41.5%, *Alternaria infectoria*—35.4% and 2 less common species *Alternaria alternata*—2.3% and *Alternaria* sp.—3.8%. In the Polissya zone in 2018, only 2 main species were identified: *Alternaria tenuissima*—31.5% and *Alternaria infectoria*—37.8%. In 2019, these species were isolated less often: *Alternaria tenuissima*—27.1%, *Alternaria infectoria*—31.4%; and фдіщ *Alternaria alternata* (0.5%) was detected.

The share of other species of fungi isolated from seeds with "black point" was much lower and ranged from 15.4 to 40.5% depending on the year and study area. Fungi of the genera *Fusarium, Epicoccum, Bipolaris* were isolated in both soil-climate zones. There was a fairly high level of grain infection by fungi of the genus *Fusarium* in Polissya, especially in 2019—10.6%. Detection of *Bipolaris sorokiniana* was recorded mainly in grains from the Polissya zone—3.5% in 2018 and 4.8% in 2019. In grains from the Forest-Steppe zone this species was detected rarely and only in 2019. In some countries, the frequency of detection of "black point" caused by fungi of the genus *Bipolaris* is at the same level as *Alternaria*, or even exceeds. In Nepal, the incidence of *Bipolaris sorokiniana* was 16.7% in Lalitpur and 64.4% in Banka [10].

The frequency of detection of *Epicoccum nigrum* ranged from 0–3.5% in the studied samples. Some species were found on grain in only one zone. Thus, *Curvularia* sp. and *Aspergillus* spp. colonized seeds only in the Kyiv region (Forest-Steppe zone). *Cladosporium herbarum*, *Aureobasidium pullulans*, *Nigrospora oryzae* were identified only in grains with "black point" from the Polissya zone. *Acremoniella atra* was found only in 2019 in seeds from both the Forest-Steppe (0.8%) and Polissya zones (1.6%).

Stemphillium vesicarium and *Sordaria fimicola* were usually found in complex on the same seed with the "black point". They were found more often in the Forest-Steppe (12.5% in 2018 and 5.3% in 2019), slightly less often in Polissya (5.2% in 2018 and 10.1% in 2019).

Stemphillium usually manifests itself as a saprophyte, and can sometimes be an optional parasite. *Sordaria* are representatives of coprophilous fungi and do not have conidial sporulation. They form dark brown or black pear-shaped perithecia in which asci with dark ascospores of color ripen [5,6].

It should be noted that causal agent of nigrospora dry rot of maize *Nigrospora oryzae* (Berk & Broom) Petch was isolated from nearly 6% of seeds from Polissya zone. This pathogen infects corn, but at high saturation of crop rotations with this crop can colonize

the seeds of cereals. *Nigrospora* was one of 11 species of fungi isolated from "black point" seeds in Paraguay [11].

5. Conclusions

In Ukraine, fungi of the genus *Alternaria* Nees prevailed in the mycobiota of wheat seeds with signs of "black point". They were found in 76.5–83.1% of seeds from the Forest-Steppe zone of Ukraine and in 59.0–69.4% of seeds from the in the Polissya zone. *Alternaria tenuissima* and *Alternaria infectoria* were found in almost the same ratio. Fungi of the genera *Fusarium*, *Curvularia*, *Bipolaris*, *Aspergillus*, *Nigrospora*, *Cladosporium*, *Acremoniella*, *Stemphillium*, *Sordaria*, *Epicoccum*, *Aureobasidium* were found to a lesser extent on seeds with black germ. The majority of prevailed fungi produce mycotoxins which are harmful for people and animals. Therefore, monitoring the species composition of pathogens and their control will ensure a high-quality harvest of wheat grain.

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